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Clinical Study Protocol

**Randomized, double-blind, placebo-controlled study to measure 2L[®]ALERG
(homeopathic drug) efficacy on symptoms of allergic rhinitis and allergic
rhinoconjunctivitis in patients with a seasonal allergy to grass pollen**

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EudraCT number : 2016-000097-38

Study acronym: LLB-2016-01

PROTOCOL INVESTIGATOR AGREEMENT

I agree:

- To conduct the study in compliance with this protocol, any mutually agreed future protocol amendments or protocol administrative changes, and with any other study conduct procedures and/or study conduct documents provided by Sponsor.
- To assume responsibility for the proper conduct of the study at this site.
- That I am aware of, and will comply with, 'Good Clinical Practice' (GCP) and all applicable regulatory requirements.
- To ensure that all persons assisting me with the study are adequately informed about the Sponsor's investigational product(s) and other study-related duties and functions as described in the protocol.
- To perform no other biological assays on the clinical samples except those described in the protocol or its amendment(s).
- To co-operate with a representative of Sponsor's designed Contract Research Organization (CRO) in the monitoring process of the study and in resolution of queries about the data.
- That I have been informed that certain regulatory authorities require the sponsor to obtain and supply, as necessary, details about the investigator's ownership interest in the sponsor or the investigational product, and more generally about his/her financial ties with the sponsor. Sponsor will use and disclose the information solely for the purpose of complying with regulatory requirements.
- To provide Sponsor with an updated Curriculum Vitae and other documents required by regulatory agencies for this study.

Study Title Randomized, double-blind, placebo-controlled study to measure 2L[®]ALERG (homeopathic drug) efficacy on symptoms of allergic rhinitis and allergic rhinoconjunctivitis in patients with a seasonal allergy to grass pollen

EudraCT number 2016-000097-38

Date of protocol 22 December 2015

Study Acronym LLB-2016-01

Investigator name

Signature

Date

SPONSOR INFORMATION SHEET

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SYNOPSIS

Study Title	Randomized double-blind, placebo-controlled study to measure 2L [®] ALERG (homeopathic drug) efficacy on symptoms of allergic rhinitis and allergic rhinoconjunctivitis in patients with seasonal allergy to grass pollen
Objectives	<p>Primary objective: To demonstrate the superiority of 2L[®]ALERG over placebo in terms of efficacy on the symptoms of allergic rhinitis and allergic rhinoconjunctivitis in patients with seasonal allergy to grass pollen, corrected according to rescue medication intake</p> <p>Secondary objectives: To compare the allergy symptoms, the rescue medication intake, the quality of life and the safety in patients treated with 2L[®]ALERG or with a placebo</p>
Study design	Multicentre, randomized, double-blind, two-parallel group, interventional placebo-controlled study Phase IV - notified homeopathic medication, marketed since 2002
Number of centres planned	20 General Practitioners (GPs)
Study Therapy	Micro-Immunotherapy medication
Planning study	Total study duration: maximum 9 months Recruitment period: 1 month Duration of patient treatment: 6 months Duration of patient follow-up : up to 8 months
Patient Population	50 patients per group to achieve 40 cases completed per group, i.e., a total of 100 patients included for 80 cases completed.
Duration of Study Follow-Up	Screening before the peak of pollination Treatment set up two months before traditional pollen peak, then visits at 3 months and 6 months, or end of the peak.
Inclusion criteria	<ul style="list-style-type: none">- Age \geq18 years, male and female- Woman of childbearing age using effective contraceptive means- Patient having the faculties to understand and respect the constraints of the study- Symptomatic since at least two seasons and confirmed by positive skin test and/or the presence of IgE for grasses (prick test defined as positive if higher than or equal to half the negative control; IgE positive if at least class 3 (\geq 3.5 kU / L); these tests must have been performed at the latest at the first screening visit- Signature of the Informed Consent Form
Exclusion criteria	<ul style="list-style-type: none">- Pregnant woman or woman wishing to become pregnant- Breastfeeding woman- Patient with an acute exacerbation of allergic rhinitis- Patient with uncontrolled asthma- Immunotherapy received within the last two years- Patient with a known lactose intolerance- Patient who participated in a clinical study in the previous three months- Patient who is not sufficiently motivated to engage on a follow-up period of 6 months or more, unable to complete the patient diary, or likely to

travel or to move before the end of the study,

- Patient taking nasal or bronchial inhaled corticosteroids on a long term basis (intermittent consumption during the season is permitted provided it is mentioned in the patient's records)

Primary endpoint

Area under the curve [AUC](total score of symptoms taking into account the Total 5 Symptom Score (T5SS) and consumption of rescue medications (RM) on the Y-axis, and time on X axis) during the entire follow-up period.

Secondary endpoints

- Total score on the T5SS scale and individual symptoms scores
- Frequency of daily RM consumption
- Evaluation of the quality of life: AUC (total score of 3 questions of quality of life included in the diary card on the Y-axis, and time on X axis) during the entire follow-up period
- Safety: adverse events (AEs) and serious adverse events (SAEs)

Sample size

A sample size of 32 patients in each group will have 90% power to detect a difference in means of 25 (the difference between a placebo group mean, μ_1 , of 80 and a 2L[®] ALERG group mean, μ_2 , of 55) assuming that the common standard deviation is 30 using a two group t-test with a 0.05 two-sided significance level.

With a sample size of 43 patients in each group, the same assumptions are valid with a common standard deviation of 35.

The final recommendation will be to include 50 patients per group in order to achieve at least 40 evaluable patients per group taking into account a potential drop-out rate of 20%.

Study cohorts

Two cohorts will be defined:

- Intention-to-treat (ITT) cohort including all patients having been exposed to at least one dose of the investigational product and in whom at least one efficacy or safety information has been collected after V0.
- Per protocol (PP) cohort including of the ITT having strictly adhered to the protocol and having a compliance between 80% and 120%.

The primary cohort for the statistical analyses will be the ITT cohort.

The patients who will prematurely stop the study will not be replaced.

Statistical analysis

A Statistical Analysis Plan (SAP) will be written by the Biostatistician of the CRO in charge of the study. This document will constitute the reference document as far as statistical analyses and statistical methodologies are concerned. It will be approved and signed by the Sponsor and the Principal Investigator before the last patient last visit at the latest.

The objective will be to demonstrate the superiority of 2L[®] ALERG over placebo. This will be demonstrated using an independent Student's test comparing the over-time T5SS (corrected with RM) evolution (AUCs) of the two study groups, over the whole treatment period. A P value lower than 5% will be considered statistically significant.

ABBREVIATIONS

AE	Adverse Event
AFMPS	Agence Fédérale des Médicaments et des Produits de Santé
AUC	Area Under the Curve
CD	Cluster Differentiation
CH	Centésimale Hahnemannienne
CRA	Clinical Research Associate
CRF	Case Report Form
CRO	Clinical Research Organization
GCP	Good Clinical Practice
GM-SF	Granulocyte-Macrophage-Stimulating Factor
HLA	Human Leukocyte Antigen
ICF	Inform Consent Form
ICH	International Conference on Harmonization
IgE	Immunoglobulin E
IL	Interleukin
ITT	Intention-To-Treat
MedDRA	Medical Dictionary for Regulatory Activities
PP	Per Protocol
RM	Rescue mediation
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAR	Serious Adverse Reaction
SmPC	Summary of Product Characteristics
SNA	Specific Nucleic Acid
SUSAR	Suspected Unexpected Serious Adverse Reaction
T5 SS	Total 5 Symptoms Score
Th	T helper
TNF	Tumour Necrosis Factor

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1. Background and study rationale

1.1. Background

For over 30 years the incidence of allergic diseases has increased so much that these diseases (asthma, atopic dermatitis, rhinitis, seasonal and annual per) present real problems of Public Health (Respiratory allergies are at the forefront of chronic diseases of the child).

The treatment of respiratory allergic diseases include the environment control, the allergen eviction (this is sometimes impossible), the use of anti-allergy medications (antihistamines, steroids, to counter the symptoms) and allergic immunotherapy (desensitization) that treats the cause.

Micro-immunotherapy, with the 2L[®]ALERG, is an alternative therapeutic approach that takes into account the patient's overall immune dysfunction, and also treats the cause, by rebalancing and re-educating the immune system.

Micro-immunotherapy medications are sequential treatments distributed in capsules, respecting the different stages of development and the evolution of the disease; they are administered sublingually, where the immunocompetent cells are concentrated (oral mucosa and neighbouring lymphoid relay), allowing a rapid transmission of the information to the lymphatic system, the meeting point and the mobilization of immune cells.

2L[®]ALERG is manufactured by the Laboratory Labo'Life Belgium, marketed since 2002 and notified to the Belgian Federal Agency for Medicine and Health Products (AFMPS /FAGG) under the number 1507 CH 119 F1.

It has been successfully used in various types of allergy, no side effects have been reported to date.

1.2. Study rationale

The aim of this study is to show the efficacy of 2L[®]ALERG on the symptoms of seasonal allergy to grass pollens, which gives allergic rhinitis and rhinoconjunctivitis. Asthma will not fit into the scope of this study.

A first randomized placebo for 2L[®]ALERG was carried out in Belgium under the leadership of Dr. X. Van der Brempt, on a population of 44 patients allergic to grass pollen, who were treated between 1 and 2 months before the pollination peak, and throughout the duration of the peak. This study showed a significant decrease in the consumption of rescue medication and in the global drug-symptoms score; it was published in 2011 in *Revue Française d'Allergologie* ^[1].

This study builds on the previous one, in order to validate this new therapeutic approach in the treatment of grass pollen allergies.

A good support to the patient is important because on the one hand, according to figures from the GA2LEN ^[2], in 2015, one European out of two suffer from some form of allergy, and on the other hand, the complications caused by the respiratory allergies are important and often underestimated. ^[3-5]. Among people with respiratory allergies, 15% to 20% suffer from a severe form ^[6-7]. The investigation of the OPERA observatory has shown that the persistent allergic rhinitis associated with moderate to severe symptoms were the most common allergic rhinitis in general practice ^[8].

Symptoms may limit daily activity, impair sleep quality ^[9] (77% of patients suffer from sleep disorders), and result in a state of fatigue, a negative impact on cognitive function, work productivity, the welfare psycho-social or on performance during exams ^[10-11]. A study has shown that students with a history of allergic rhinitis with hay fever symptoms had twice more bad exam results ^[12].

Experts recommend early introduction of the most global possible treatment, to treat but also to prevent the onset and/or worsening of allergy and asthma ^[13-18].

The different therapeutic options, apart from the environmental control and allergen avoidance (which is sometimes impossible) are the anti-allergy medications (antihistamines, steroids, to counter the symptoms) and the allergic immunotherapy (desensitization) that treats the cause, and therefore the symptoms ^[19-22]. Allergen immunotherapy is specific to a definite allergen and is administered mainly by subcutaneous or sublingual route, rarely by nasal route ^[23-24], the average duration of treatment is 3 to 5 years and involves a rigorous monitoring of protocol, to be effective [25], making it constraining treatment, and sometimes not suitable.

Micro-Immunotherapy ^[26], with 2L[®]ALERG, is an interesting alternative therapeutic approach for taking into account the patient's overall care, preventive as well as curative.

Let's see how 2L[®]ALERG can solve the problem, first let's see what the actors of an allergic reaction are.

The allergic reaction is the result of an inappropriate immune response of the body, following the meeting with a foreign substance, the allergen. Allergy can affect the eyes, skin and respiratory system, like the nose or bronchi.

The most common mechanism, at the origin of rhinitis, conjunctivitis and allergic asthma, is immediate hypersensitivity.

As in all hypersensitivity responses, there are two phases in immunological immediate hypersensitivity. A first phase of sensitization/immunization, during which the immune system identifies the substance as an allergen, leading to the synthesis of IgE. It is clinically silent. The second phase, called "revelation" or "effector", when the body again comes into contact with the allergen, it is clinically symptomatic, due to the immediate activation by allergen of cells bearing IgE on their surface (mainly mast cells and basophils).

The tissue borders of the body (such as skin or the respiratory or digestive mucosa) are the interface between the environment and the immune system and have a dual function of defence against infection and maintenance of tolerance with respect to environmental antigens. Depending on the size or nature of the antigen, the tissue borders prevent the penetration of antigens, or, if penetration occurs (e.g., in the digestive tract), keep and maintain tolerance through different mechanisms.

Dendritic cells, present in all tissue borders, permanently capture antigens that enter. Dendritic cells having internalized the antigens, migrate to loco-regional lymph nodes completing their maturation. They then induce a cellular immune response by interacting with T cells. B cells specific response is also set up with the help of T helper lymphocytes, which results in IgE production by plasma cells.

This excessive IgE production is related to a particular cytokines environment:

- Rich in IL-4, IL-5 and IL-13 (called hyperpolarization Th2)
- With a relative deficiency in IL-10 and TGF (Tregs default)
- Deficit in γ interferon (Th1 polarization default)

This particular profile of cytokine production (**IL-4, IL-5, IL-13**) is also responsible for an increase in the number of mast cells, basophils and eosinophils into the tissues or the blood. Produced IgE present themselves in the circulating blood and in tissues, free or attached to the surface of mast cells and basophils. IgE persist for several months at the cell surface but only a few days in free form in the peripheral blood.

All of these processes correspond to the immunization phase or primary immune response, called sensitization in the case of allergy, resulting in IgE production against a particular antigen. The IgE production against a given protein is polyclonal. The sensitization phase can last from several weeks to several years.

The effector phase of the IgE-dependent immediate hypersensitivity is mainly the preservation of mast cells. In some cases, basophils may also play this role. The most important function of mast cell in the pathology is the effector phase of the allergic response.

After the sensitization described above, in case of a new contact with the allergen, the recognition of the same allergen, by IgE that are on the surface of mast cells or basophils, leads to the cascade activation of these cells.

The secretory granules contain numerous preformed mediators of the immediate phase vasoactive: amines (histamine mainly), proteoglycans, polypeptides, lysosomal enzymes, cytokines and chemokines.

A mast cell can release at once 100% of its content of secretory granules and can participate in multiple episodes of degranulation, with a regeneration time of the granulations of 72 hours. This explains the need to respect a delay between the occurrence of an allergic episode and achievement of in vivo tests (skin tests) or ex vivo (measurement of basophils activation) to determine the aetiology of this episode.

During exocytosis of granular contents, histamine diffuse through the tissues to bind to its receptors and cause vasodilation and increased capillary permeability (clinical consequences: hives, deep tissue oedema, circulatory failure up to anaphylactic shock), bronchospasm (consequence: asthma), hypersecretion of bronchial mucus. The released proteases, such as tryptase, NO synthase and beta-hexosaminidase, initiate inflammation and local tissue degradation. Chemokines attract and activate leukocytes, supplemented by the effect of the preformed cytokines TNF-alpha and IL-4, which release directs from the beginning to a new local immune response towards Th2 arm.

After this immediate phase of the effector phase, comes the second step: the mast cell activation through stimulation of FcεRI receptor, which indeed leads not only to rapid exocytosis of granular contents, but also to the deferred production of neoformed mediators. A wide range of these mediators can be produced, but the secretion is adapted to the type of stimulus received by the mast cell and its environment.

Among the cytokines and mast cell growth factors neosynthesized the most important we find pro-inflammatory agents (**IL-1, TNF, IL-6**), cytokines involved in the orientation of Th responses (**IL-10, IL-4, IL-13**), **IL-5** that promotes the recruitment and activation of eosinophils, the Stem Cell Factor, main mast cell growth factor, GM-CSF growth factor of myeloid cells. Together, these newly formed mediators, often on an autocrine mode, contribute to the installation of a chronic inflammatory reaction with tissue remodelling (e.g., airway

remodelling in asthma, remodelling of the nasal mucosa in chronic rhinitis). The resolution failure is common and leads to the occurrence of irreversible anatomical lesions, self-sustained. Beside the mast cells, many other cells are involved in the pathogenesis of immediate hypersensitivity. Eosinophils (containing many mediators within their granules), basophils and neutrophils attracted on the site of the reaction through soluble mediators, perpetuate the lesions. The role of basophils may be dominant in the orientation Th2 immune responses on contact with the allergen.

Genetic factors are important, in fact, several susceptibility genes have been associated with allergic diseases:

- HLA molecules (ensuring the presentation of allergens to CD4 lymphocytes)
- B chain for the high-affinity receptor for IgE (FcεRIβ)
- IL-4
- Receptor beta - adrenergic
- TNF
- CD14 (co-receptor lipopolysaccharide)

Genetic changes may explain the increase in allergies in recent decades, however, it is now recognized that gene transcription is modulated by the environment (defining "epigenetic").

Let's now see how the 2L[®]ALERG, Micro-Immunotherapy medication, can act:

Micro-Immunotherapy is based on the use of endogenous molecules obtained by biotechnological synthesis and administered in very low doses sublingually. These physiological mediators aim to restore the balance Th1/Th2 ^[27] without focusing on the causative agent of the disease.

Composition of 2LALERG[®]:

Interleukin 1	17 CH
Interleukin 4	17–27 CH
Interleukin 5	17 CH
Interleukin 6	17 CH
Interleukin 10	17 CH
Interleukin 12	9 CH
Interleukin 13	17 CH
Tumor Necrosis Factor Alpha	17 CH
Transforming Growth Factor Beta	5 CH
Pulmo histaminum	15 CH
SNA-HLA-II	18 CH

According to the law of Arndt-Schultz, high dilutions are used to obtain a braking effect, average dilutions, a modulating effect, and low dilutions, a stimulating effect.

IL-1 is in high dilution to slow down the activity of B and T lymphocytes, protein production and the acute phase of inflammation, and IL6 production by Th2 ^[27-29].

IL-4 is in high dilution to reduce Th2 differentiation ^[30] and to brake IgE synthesis.

IL-5 is high dilution to slow activation and proliferation of eosinophils and thus prevents the influx into the airways.

IL-6 is in high dilution to slow the inflammatory Th2 response ^[31] under the influence of IL-1.

IL-10 is in high dilution to reduce the stimulatory effects of IL-4 ^[32].

IL-13 is in high dilution to decrease the expression of HLA class II molecules on macrophages and to brake the production of IgE [33], which reduces the allergic reaction.

TNF- α in the high dilution, coupled with high dilution of IL-1, tends to reduce the recruitment of eosinophils in the inflammatory site (located at the ENT or bronchial mucosa).

The **SNA-HLA-II** is in high dilution to directly decrease the expression of HLA class II molecules.

TGF- β is in low dilution to increase the inhibition of cytokine production and the expression of class II molecules.

IL-12 is on average dilution to modulate the differentiation of T lymphocytes of Th1 type [34], to compensate for the antagonistic effect of IL-4 and IL-10, IL-12, and to reduce probable mucosal alterations in bronchopulmonary level.

Our objective is now to confirm the efficacy of 2L[®] ALERG, highlighted in the previous study [1], which results were the basis of the actual calculation for this study.

X. Van der Brempt et al. / Revue française d'allergologie 51 (2011) 430–436: Pollen count and over-time modification of the global score (mean and standard deviation) in the active and placebo groups during the study period.

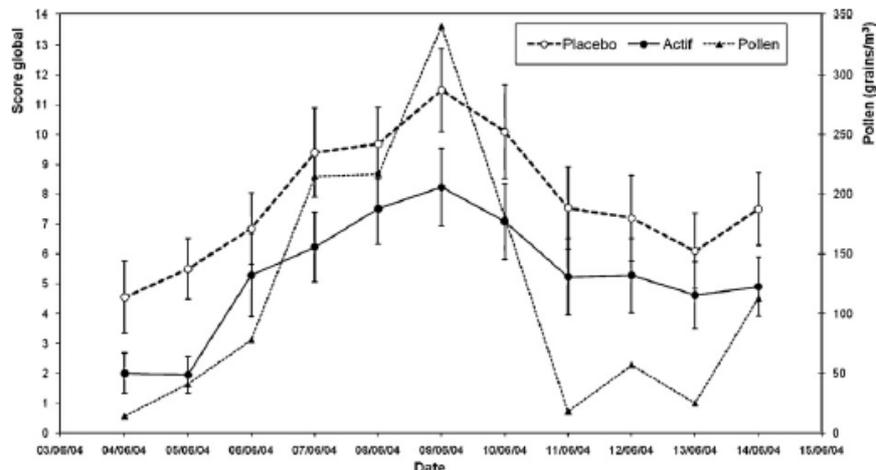


Fig. 5. Comptages polliniques et évolution du score global (moyenne et écart-type) dans les groupes actif et placebo pendant la période d'étude.

Patients suffering from grass pollen allergy will be randomized into 2 groups: a control group and a treatment group.

The evaluation will focus on the 5 symptoms of allergic rhinitis/rhino-conjunctivitis assessed daily by patients on a scale from 0 (no symptoms) to 3 (severe symptoms) for a total score ranging from 0 to 15 (T5SS or Total 5 Symptoms Score) [35-36].

Rescue medication (RM) will be allowed in case of failure or insufficient treatment. The use of rescue medication will be recorded daily by the patient in his/her diary.

Given the link between the use of rescue medication and the symptoms, an overall score will be calculated, expression of symptoms score, weighted by rescue medication score, according to the methodology currently advocated for the development of anti-allergy medications. [35-37]

1.3. Benefits and risks for people involved in the study

Benefits

The person who participates in research can expect a personal benefit.

This benefit will be objectified by decreased symptoms, a decreased consumption of rescue medication, and an improved quality of life.

Risks

Given the type of study medication, its perfect tolerance, given that the study does not require any invasive procedure, and that rescue medication is allowed, there is no potential risk for the patients.

To date 98,000 boxes of 2L[®]ALERG were sold and no side effects have been reported.

Risk-benefit balance

Given the potential impact on the quality of life of patients and given that no side effect are expected with 2L[®]ALERG, the balance is strongly in favour of the benefit, and without any potential risk for patients who may use rescue medication.

1.4. Description and justification of the therapeutic schema

The study drug is 2L[®]ALERG.

The treatment consists of taking one capsule a day, before breakfast, following the order of numbering: 1 through 10. When the number 10 has been taken, start another blister on the next day.

Method of administration: open the capsule, pour the contents (granules) under the tongue and let it dissolve. The pellets will be taken on an empty stomach, 15-30 minutes before the morning meal, or if taking has been forgotten, one hour after the meal.

The duration of treatment will be about 6 months.

2. Objectives and endpoints

2.1. Primary objective and primary endpoint

The main objective is to demonstrate the efficacy of 2L[®]ALERG versus placebo on the symptoms of allergic rhinitis and allergic rhino-conjunctivitis in patients with seasonal allergy to grass pollen.

The primary endpoint will be the area under the curve (AUC) of the overall score established according to the total 5 symptoms score (T5SS) and medication score (MS) according to time from the start of treatment until the end of the patient follow-up.

The five symptoms (sneezing, rhinorrhoea, nasal pruritus, eye itching and tearing, and nasal obstruction) will be assessed daily by patients on a scale from 0 (no symptoms) to 3 (severe symptoms) giving a total score ranging from 0 to 15.

The rescue medications (RM) allowed in the first-line adjuvant treatment will be codified to establish a score.

The allowed RM are the oral antihistamines (two points per day of use) or local treatment (nasal or eye; a point per day), and the ocular cromoglycate (one point per day).

In case of failure or in case of insufficiency of rescue medications mentioned above, the nasal topical corticosteroids (one point per day of use) will be allowed.

2.2. Secondary objectives and secondary endpoints

The secondary objectives will be to compare the effect of 2L[®]ALERG versus placebo on:

- Symptoms of allergy (not corrected by the use of rescue medication),
- Use of rescue medication,
- Evolution of the quality of life (QoL) using 3 daily specific questions included in the patient's diary card: Did you sleep well? – Can you work normally? – How do you feel?
- Safety (AEs and SAEs).

The secondary endpoints will be:

- Total 5 symptoms score (T5SS) and individual scores of 5 symptoms
- Consumption of rescue medications
- Evaluation of the QoL: AUC (total score of 3 questions of QoL included in the diary card on the Y-axis, and time on X axis) during the entire follow-up period
- Frequency, severity and causality relationship of AEs and SAEs.

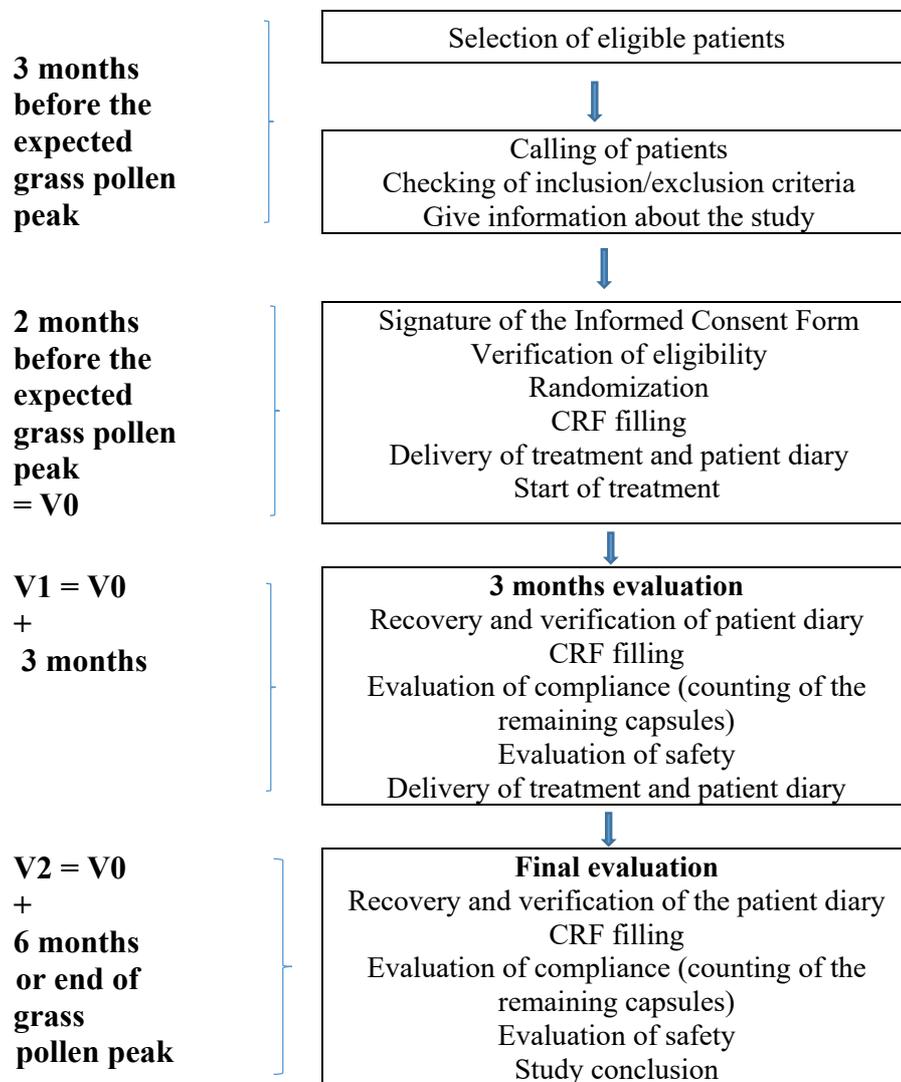
3. Study design

3.1. Methodology

It is a double-blind, randomized, placebo-controlled, 2 parallel groups, interventional study, performed with a notified homeopathic medicine, available on the market since 2002.

3.2. Study design

- The study will last maximum 9 months: duration of inclusion = 1 month and patient's follow up = 6-8 months.
- The duration of 2L[®]ALERG exposure will be 6 months.



4. Study population

4.1. Description of the population

Hundred patients to achieve 80 completed cases (50 patients per group to achieve 40 completed cases).

4.2. Inclusion criteria

- Age ≥ 18 years, male and female
- Woman of childbearing age using effective contraceptive means
- Patient having the faculties to understand and respect the constraints of the study
- Symptomatic since at least two seasons and confirmed by positive skin test and/or the presence of IgE for grasses (prick test defined as positive if higher than or equal to half the negative control; IgE positive if at least class 3 (≥ 3.5 kU / L); these tests must have been performed at the latest at the first screening visit
- Signature of the Informed Consent Form

4.3. Exclusion criteria

- Pregnant woman or woman wishing to become pregnant
- Breastfeeding woman
- Patient with an acute exacerbation of allergic rhinitis
- Patient with uncontrolled asthma
- Immunotherapy received within the last two years
- Patient with a known lactose intolerance
- Patient who participated in a clinical study in the previous three months
- Patient who is not sufficiently motivated to engage on a follow-up period of 6 months or more, unable to complete the patient diary, or likely to travel or to move before the end of the study,
- Patient taking nasal or bronchial inhaled corticosteroids on a long term basis (intermittent consumption during the season is permitted provided it is mentioned in the patient's records)

5. Study drugs and concomitant medications

5.1. Description of the study drugs and manufacturing process

The composition of 2LALERG[®] and placebo are described in the table below:

	2LALERG[®]	PLACEBO
Impregnated solution:	Interleukin 1: 17 CH Interleukin 4: 17-27 CH Interleukin 5: 17 CH Interleukin 6: 17 CH Interleukin 10: 17 CH Interleukin 12: 9 CH Interleukin 13: 17 CH Tumor Necrosis Factor Alpha: 17 CH Transforming Growth Factor Beta: 5 CH Pulmo histaminum: 15 CH SNA-HLA-II: 18 CH	No impregnation
Excipients :	Lactose saccharose globules (380mg/capsule)	Lactose saccharose globules (380mg/capsule)
Primary packaging :	Red capsules (color degradation ranging form dark red to pink, 5 colors)	Red capsules (color degradation ranging form dark red to pink, 5 colors)
Secondary packaging :	Aluminium sealed blister of 10 capsules numbered from 1 to 10, Labelled 2LALERG-PLACEBO 10A16-15A16	Aluminium sealed blister of 10 capsules numbered from 1 to 10, Labelled 2LALERG-PLACEBO 10A16-15A16
Final packaging :	Printed cardboard box (see annex 1) containing 3 blisters and one leaflet explaining how to take the medicine (see annex 2)	Printed cardboard box (see annex 1) containing 3 blisters and one leaflet explaining how to take the medicine (see annex 2)

The manufacturing process consists of 4 steps:

STEP 1: DILUTION PREPARATIONS

STEP 2: IMPREGNATION

STEP 3: GLOBULES ENCAPSULATION: PRIMARY PACKAGING

STEP 4: SECONDARY PACKAGING and FINISHED PRODUCT PACKAGING

The placebo is manufactured exactly as the 2LALERG[®], except that it contains only the neutral globules without the homeopathic dilutions impregnated on it. The capsulation, blisterisation and final packaging process are exactly identical for both.

5.2. Administration of study drugs

Treatment used: 2L[®]ALERG - Placebo

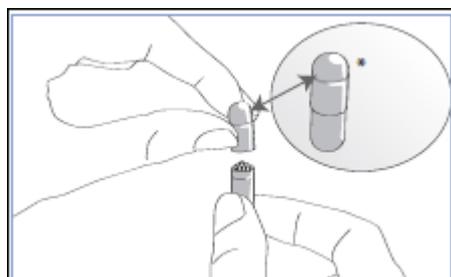
Pharmaceutical form: Capsules - doses containing impregnated granules

Excipients: Lactose, Sucrose (granules) to a capsule.

Dosage: 1 capsule daily, fasting morning, following the numerical order of 1 to 10 capsules. When capsule 10 has been taken, start the next blister at capsule 1.

Administration mode:

Open the capsule, pour the contents (granules) under the tongue and let it dissolve. The pellets will be taken on an empty stomach, 15-30 minutes before the morning meal, or one hour after the meal if forgotten.



1. Open carefully the capsule maintaining the capsule's head upward



2. Pour the content (granules) under the tongue and allow melting

Contraindications: None

Warnings and precautions:

This medicine contains lactose. Lactose intolerance cases have been described. The amount of lactose present in the capsules is theoretically sufficient to trigger symptoms of intolerance. In cases of outbreaks of diarrhoea, the patient should consult his/her doctor.

As with any homeopathic medicine, it is advisable to avoid taking mint or its derivatives and stimulant products (coffee, chocolate) during one hour before the intake.

Adverse reactions (frequency and seriousness)

Exceptionally, it may appear dyspepsia when taken during fasting. In this case, the granules will be taken one hour after the meal.

Difficulties falling asleep can occur when taken after 16 hours.

As with all homeopathic treatment, worsening of symptoms printing can occur, which usually disappears after a few days. If this impression persists, a physician should be contacted.

Dosage adjustment: Not applicable

5.3. Permitted and prohibited drugs and treatments

Allowed treatments:

- Already established treatments for associated pathologies not liable to have an impact on the proper conduct of the study,
- Rescue medication allowed in the first-line adjuvant treatment is: oral or topical antihistamines (nasal or eye) and eye cromoglycate, topical nasal corticosteroids (in case of failure or insufficiency of those above).

Not allowed treatments:

- Oral or injectable corticosteroids
- Anti-leukotrienes

5.4. Randomization and blinding

The randomization list will be prepared by a Data Manager (independent from the clinical team involved in the study) from the Data Management Department of the CRO, using the randomization module of nQuery Advisor (Version 7.0), ensuring a ratio 1:1 between the study drug and the placebo in each centre.

The investigator of each centre will assign treatment in an ascending order to his/her patients.

The study will be double-blind, meaning that nor the investigator, nor the patient will know the treatment group (active or placebo) to which the patient belongs. The study drug and the placebo will have exactly the same aspect, form, odour and colour. Their packaging will also be exactly the same.

5.5. Monitoring of compliance

The drugs will be sent to investigators (GPs in private practice).

The treatment beginning two months before the expected grass pollen peak, the investigator will deliver it to the patient for 3 months (3 boxes) at V0, then for three other months at V1.

The investigator will ask the patient to bring back drug boxes (full and empty blisters) at each visit.

The counting of the remaining capsules will be made by the investigator.

In case the patient would come in consultation a few days before the end of the treatment period, the patient will be allowed to keep the blister to complete the sequence.

A global compliance will be calculated for each patient on the total duration of treatment. Global compliance will be acceptable if it is between 80% and 120%. If values <80% or > 120%, the patient will be eliminated from the analysis according to the protocol and will be taken into account in the analysis by intention to treat.

5.6. Labelling and packaging of investigational medicinal products

The sponsor Labo'Life will provide drugs to investigational centres.

The labelling and packaging will be handled by Labo'Life according to Good Manufacturing Practice Annex 13.

At the end of the study, Labo'Life will recover the remaining treatments and ensure their destruction.

Drug storage conditions

- In the investigational centre: at room temperature, in its original packaging and in a locked cabinet.
- At patient's home: at room temperature, in its original packaging and out of reach of children.

6. Conduct of the study

6.1. Study procedures

The following demographic data will be collected during the first visit: date of birth, gender, ethnic group, weight, height, smoking habits and alcohol use status.

Medical history, treatment history, concomitant treatments and patient's physical examination will also be documented during V0.

The allergy must be confirmed by positive skin test and/or the presence of IgE for grasses (prick test defined as positive if higher than or equal to half the negative control; IgE are positive if at least class 3 (≥ 3.5 kU / L); these tests must have been made at the latest during the first visit.

A patient diary will be given to each patient.

- For evaluation of the total 5 symptoms score T5SS and rescue medication RS, it is essential that patients note **every day** the symptoms (sneezing, rhinorrhoea, nasal pruritus, itching and/or eye tearing and nasal obstruction) intensity, and the rescue medications, in their patient diary.
- It is also essential that the patient takes note **every day** of his/her QoL by filling in three questions included in his/her diary card: Did you sleep well? – Can you work normally? – How do you feel?
- It is also imperative that patients note the date of the start of treatment and the date of onset of symptoms.

This patient diary will be given to the patient at each visit, and will be brought back at the next visit, it will be reviewed and validated by the investigator and will be part of the CRF (Case Report Form).

All relevant (related to allergy) concomitant medications (except rescue medications) taken by the patient during the study period will be recorded at each visit in the CRF.

The AEs and SAEs will be recorded at V1 and V2 in order to assess safety.

6.2. Study schedule

Study flow chart

	Screening (- 1 month)	V0 (2 months before grass pollen peak)	V1 (3 months after V0)	V2 (6 months after V0 or end of grass pollen peak)
Selection of patients Verification of inclusion/exclusion criteria Performance of allergy tests if needed	●			
Give information about the study Give the Inform Consent Form to the patient	●			
Eligibility check		●		
Signature of the Informed Consent Form		●		
Randomization		●		
Demography		●		
Medical history		●		
Physical examination		●	○	○
Allergy treatment history		●		
Delivery of treatment for 3 months		●	●	
Distribution of the patient diary		●	●	
Start of treatment		●		
Diary card filling by the patient		●	●	●
Patient diary card control			●	●
Compliance control			●	●
Relevant (allergy-related) concomitant medications (except rescue medications)		●	●	●
Safety control (AE/SAE)			●	●
Study conclusion				●

- Compulsory
- Optional

6.3. Identification of all the data sources that are not in the medical file

All data collected in the CRF have to be recorded in the patient medical file.

Patient diary data are source data which will be directly captured by the data management.

6.4. Rules for study discontinuation

Criteria for premature discontinuation of the study

- Withdrawal of consent,
- Protocol violation,
- Intolerance to treatment,
- Voluntary discontinuation of treatment,
- Intake of a forbidden treatment during the study,
- Proved pregnancy,
- Decision at the discretion of the investigator.

Criteria for stop the study

The study will be considered terminated at the end of the participation of the last patient entered into the study, and when all collected data needed for evaluation have been verified and validated.

7. Data Management and statistics

7.1. Data collection and handling of study data

Data collection

Data collection will be done on paper CRF for each patient, The patient will fill in the patient diary card given by the investigator and bring it back at the next visit. The investigator will check the patient diary card and will keep it in the patient CRF The sponsor will be responsible for the printing of CRFs (NCR carbon paper) and patient diaries, which will be sent to each investigator. The persons allowed to fill in the CRF are identified in the delegation of responsibilities sheet which is to be kept in the Trial Master File.

Data coding

By signing this protocol the investigator and all the staff are committed to keep confidential the identities of the patients participating in the study.

Each patient will be identified by a numerical code consisting of 4 digits: YY/ZZ (YY corresponding to the centre number and ZZ to the patient number).

A form will be made available to the investigator so he could note the correspondence between the numeric code and the patient's identity. He/she must keep this document in his/her Trial Master File.

Collection Data Processing

The compilation of clinical data will be based on the structuration of a clinical database (IBM SPSS Data Entry Builder) and the creation of input screens in the image of CRF and diary card in accordance with the Protocol and the legislation currently in force.

This database will be validated and documented appropriately.

All data will undergo a double data entry process in the database, by two independent encoders, using the IBM SPSS Data Entry Interviewer software.

Based on the regular cleaning of data, queries will be generated to the investigational centres. The answers to these queries will also be encoded in the database.

7.2. Biostatistics

Sample size calculation and justification

The objective of the study will be to show the superiority of 2L[®]ALERG over placebo in the treatment of patients suffering from seasonal allergy to grass pollen.

For the sample size calculations two references have been used. The first is a study conducted with 2[®]LALERG versus placebo in patients with a pollen allergy. ^[1]

The second is a more theoretical publication from Clark and Schall (2007) supporting the recommendations made in a World Allergy Organization document on methodological aspects of immunotherapy trials. The average of the Average Rhinoconjunctivitis Total Symptom Score (ARTSS) and Average Rescue Medication Score (ARMS) should be considered as a primary efficacy variable in clinical trials of immunotherapy for allergic rhinoconjunctivitis. ^[35]

The global score calculated in the paper of Van der Brempt et al. (2011) [1], taking into account the T5SS total score and the RMS total score, fits very well with the recommendation from Clark and Schall (2007) [35].

The Figure 5 of Van der Brempt et al.'s paper has been used to evaluate the area under the curve (AUC) of global score for the placebo and 2L[®] ALERG groups. [1]

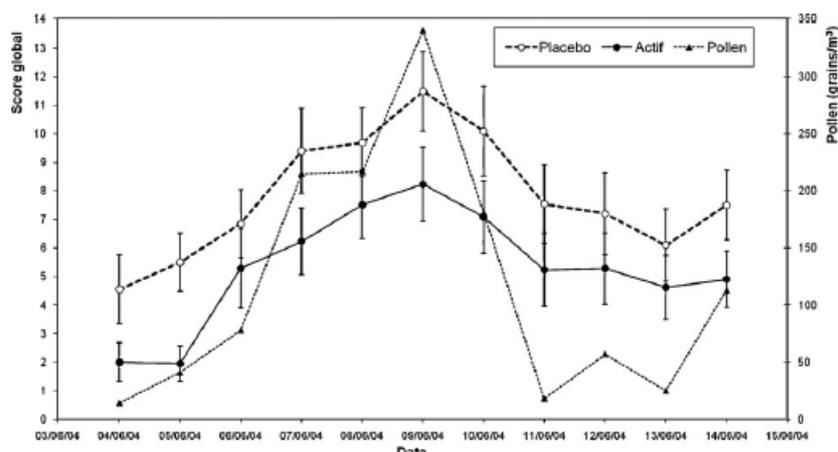


Fig. 5. Comptages polliniques et évolution du score global (moyenne et écart-type) dans les groupes actif et placebo pendant la période d'étude.

The values were approximately 80 for the placebo group and 55 for the treated group (a reduction of about 30% with the active treatment). These values have been used to build several sample size scenarios.

Scenario	1	2	3	4	5
Test significance level, α	0.050	0.050	0.050	0.050	0.050
1 or 2 sided test?	2	2	2	2	2
Group Placebo mean, μ_1	80.000	80.000	80.000	80.000	80.000
Group 2L [®] ALERG mean, μ_2	55.000	55.000	55.000	55.000	55.000
Difference in means, $\mu_1 - \mu_2$	25.000	25.000	25.000	25.000	25.000
Common standard deviation, σ	20.000	25.000	30.000	35.000	40.000
Effect size, $\delta = \mu_1 - \mu_2 / \sigma$	1.250	1.000	0.833	0.714	0.625
Power (%)	90	90	90	90	90
n per group	15	23	32	43	55
Total N completed patients	30	46	64	86	110

A sample size of 32 patients in each group will have 90% power to detect a difference in means of 25 (the difference between a placebo group mean, μ_1 , of 80 and a 2L[®] ALERG group mean, μ_2 , of 55) assuming that the common standard deviation is 30 using a two group t-test with a 0.05 two-sided significance level.

With a sample size of 43 patients in each group, the same assumptions are valid with a common standard deviation of 35.

The final recommendation will be to include 50 patients per group in order to achieve at least 40 evaluable patients per group taking into account a potential drop-out rate of 20%.

Statistical analyses

A Statistical Analysis Plan (SAP) will be written by the Biostatistician of the CRO in charge of the study. This document will constitute the reference document as far as statistical analyses and statistical methodologies are concerned. It will be approved and signed by the Sponsor and the Principal Investigator before the last patient last visit at the latest.

Study cohorts

Two cohorts will be defined:

- Intention-to-treat (ITT) cohort including all patients having been exposed to at least one dose of the investigational product and in whom at least one efficacy or safety information has been collected after V0.
- Per protocol (PP) cohort including of the ITT having strictly adhered to the protocol and having a compliance between 80% and 120%.

The primary cohort for the statistical analyses will be the ITT cohort.

The patients who will prematurely stop the study will not be replaced.

Statistical methods

The IBM SPSS Statistics (Version 21.0 and eventual upgrades) software will be used throughout statistical analyses.

Missing values will not be replaced, nor extrapolated.

Classical descriptive statistics will be used to describe the patient population at baseline.

The two groups will be compared at baseline using independent Student's t tests for continuous variables and Mann-Whitney's tests, Chi-square tests or Fisher's exact tests for discrete variables.

The superiority of the active treatment over placebo will be demonstrated using an independent Student's test comparing the over-time T5SS (corrected with RM) evolution (AUCs) of the two study groups over the whole treatment period. A P value lower than 5% will be considered statistically significant.

The comparisons between the two study groups in terms of secondary endpoints will be done using the appropriate statistical methods, as they will be described in the SAP. No correction being applied for the multiplicity of endpoints, p values lower than 5% will be considered with caution, as indicative only of potential statistically significant differences between the two groups for the secondary endpoints.

8. Pharmacovigilance and management of adverse events

8.1. Definitions

Adverse events

An adverse event is defined as any untoward medical occurrence in a patient or a participant in a clinical trial, whether or not considered drug related.

All adverse events encountered during the study, which are observed by the physician or patient-reported, will be recorded in the CRF in the section provided for this purpose.

The intensity of adverse events will be quoted as follows:

- 1 = mild
- 2 = moderate
- 3 = severe

For each adverse event, the investigator will have to rule on the causal relationship to the medical product on the following scale: none, possible, probable or certain.

Adverse events or serious adverse events (AEs or SAE)

An AE is considered as SAE when it:

- Results in death,
- Involves life-threatening,
- Results in incapacity or temporary or permanent disability,
- Requires or prolongs patient hospitalization,
- Causes congenital anomaly or neonatal
- Is medically significant (requires care to avoid worsening)

Serious Adverse Reaction (SAR)

A SAR is a SAE considered related to the investigational study product.

Expected adverse reaction or events

An expected adverse event is an event mentioned in the most recent version of the Summary of Product Characteristics (SmPC) for drugs already having a marketing authorization.

The expected effects or serious adverse events will be subject to a delayed declaration by the sponsor to the competent authorities.

Unexpected Serious Adverse Reactions (SUSARs)

Unexpected Serious Adverse Reactions (SUSARs) are events where the nature, severity, frequency or developments are not consistent with product information, with practiced acts and with methods used during the study, as defined in the SmPC.

Unexpected serious adverse events will be subject to a statement within 7 days after their knowledge by the sponsor to the competent authorities.

8.2. Safety endpoints

Collecting data and analysis of safety endpoints described above will be performed by spontaneous statement or during monitoring.

An SAE report form will be attached to the CRF (collecting symptoms, dates, evolution, judgment of causality by the physician, actions taken)

All AEs / SAEs / SARs / SUSARs will be subject to coding in terms of System Organ Class and Preferred Terms, using the latest version of the dictionary MedDRA (Medical Dictionary for Regulatory Activities).

8.3. List of expected AEs

In the framework of this study, expected AEs are those mentioned in the SPC:

- In exceptional cases, it may appear dyspepsia when taken fasted (in this case, the granules will be taken one hour after the meal)
- Difficulties in falling asleep can occur if taken after 16 hours
- As with all homeopathic treatment, print of worsening symptoms can occur, that usually disappears after a few days.

8.4. Management of adverse events

All SAEs require filling a SAE report, whether expected or not expected. The investigator must ensure that the information entered on this report is accurate and clear.

SAE should be reported immediately to the sponsor (within 24 hours of being highlighted by the investigator).

After being scanned, the SAE report can be sent by e-mail to the following person in charge of pharmacovigilance:

Dr. Paul Willems
Semaphar sprl
31, Rue Francourt
1370 Lathuy (Jodoigne)
Belgium
Mobile: 0476/777.987
E-mail address: pwillems.md@icloud.com

The person in charge of pharmacovigilance will have a copy of the randomization list available in case unblinding is unavoidable after his/her discussion with the investigator.

After receipt of notification of a SUSAR, the sponsor shall declare to the regulatory authorities. Once a year, the sponsor will prepare an annual safety report.

8.5. Modalities and duration of follow up after the occurrence of adverse events

Given the nature of the study treatment, the follow up shall be two months after the end of the study.

9. Administrative and regulatory aspects

9.1. Right of access to source data

The medical data of each patient will only be transmitted to the sponsor or to any person duly authorized by him, and, as appropriate to relevant health authorities, under conditions ensuring their confidentiality.

The sponsor and the regulatory authorities may request direct access to medical records for verification of procedures and/or clinical trial data, without violating confidentiality and as permitted by laws and regulations.

The data collected during the test will be subject to computerized processing in accordance with the requirements in place in Europe.

9.2. Monitoring

Clinical monitoring will be ensured by the CRO.

A Clinical Research Associate (CRA) will regularly visit the site to carry out quality control of data reported in the CRFs.

The on-site monitoring visits will be organized after appointment with the investigator. The CRA will be allowed to consult:

- CRFs
- Patient's diary cards
- Medical files
- Trial Master File.

9.3. Inspection / Audit

In the context of this study, an inspection or an audit by the regulatory authorities may take place.

The sponsor also reserves the right to audit the investigational centres, either itself or by a third person appointed by him.

9.4. Ethical considerations

Written informed consent:

The entire informed consent process involves giving a participant adequate information concerning the study, providing adequate opportunity for the participant to consider all options, responding to the participant's questions, ensuring that the participant has understood this information, obtaining the participant's voluntary agreement to participate, and continuing to provide information as the participant or situation requires. To be effective, the process should provide ample opportunity for the investigator and the participant to exchange information and ask questions.

The investigator undertakes to inform the patient clearly and after taking time for reflection the patient will sign and date the Informed Consent Form (ICF). The investigator must also sign and date the ICF.

Both documents will be issued on paper in 2 copies so the patient and the investigator can each keep a copy. The original one is for the investigator and will be archived in the Trial Master File.

Ethics Committee:

The sponsor undertakes to submit the study protocol, the diary card and the EQ-5D scale to the prior approval of the Ethics Committee.

Amendments to the protocol: change requests will be sent for authorization in accordance with current legislation, by the sponsor, to the competent authorities. The modified protocol will then undergo an updated version dated.

9.5. Declaration to the competent authorities

An EudraCT application will be submitted to the regulatory authorities (AFMPS/FAGG).

9.6. Financing and insurance

The sponsor shall finance the study and an insurance policy covering the financial consequences of its civil liability in accordance with the regulations.

9.7. Publication rules

The data collected in this study are the property of the study Sponsor, no publication can be made without its agreement.

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